Systemic administration of a novel development candidate, MTL-CEBPA, upregulates the liver-enriched transcription factor C/EBP-α and reverses CCl4-induced liver failure in vivo

Reebye V¹, Voutila J², Huang K³,⁴, Muragundla A⁵, Jayaprakash A⁵, Vadnal P⁵, Huber H⁶, Habib R², Saetrom P⁷,⁸, Rossi J⁹, Habib N¹*
MTL-CEBPA upregulates C/EBP-α by transcriptional activation

1. Loading of saRNAs into Ago2 protein
2. saRNA-Ago2 recruit transcription complexes
3. Promoter remodelling
4. Long lasting protein up-regulation
C/EBP-α is an attractive target in liver disease

C/EBP-α transcription factor

- Master regulator
  - cell lineage determination
  - cell growth and proliferation
  - maintaince of metabolic balance and body weight homeostasis

- Essential role in hepatocytes
  - differentiation
  - lipid and glucose homeostasis

Rationale for upregulation in liver disease

- Dysregulated in major liver diseases, including NAFLD, NASH, and HCC
- Overexpression reduced fibrosis in mice
- Up-regulating improved liver function in DEN model of cirrhosis and HCC in rats

MTL-CEBPA dosed in CCl4 model of liver failure

- Hepatic fatty infiltration
- Fibrosis
- Liver injury
- Impaired liver function

Sprague Dawley rats
Male at 6-7 weeks
n = 9

- CCl4 dosing (Weeks 1 – 10)
- Treatment (Weeks 8 – 10)

Humanely kill
BIW x2 i.v. at 3mpk
MTL-CEBPA restores CEBPA mRNA in cirrhotic liver

![Graph showing normalized CEBPA mRNA levels across different conditions.](image-url)
MTL-CEBPA normalises liver hydroxyproline

Hydroxyproline (μg/g liver)

- Normal control
- Path control (Week 8)
- NOV340 +siFLUC
- MTL-CEBPA

+CCI4 dosing

***
Reduced fibrosis and fatty infiltration in MTL-CEBPA treated animals at week 10

NOV340 + siFLUC

MTL-CEBPA
MTL-CEBPA normalises serum bilirubin

**Pre treatment**
Week 8

**Post treatment**
Week 10

![Graph showing bilirubin levels before and after treatment](image)

- **Normal control**
- **NOV340 + siFLUC**
- **MTL-CEBPA**

**+CCI4 dosing**

- **Normal control**
- **NOV340 + siFLUC**
- **MTL-CEBPA**

***P-value indicates significant difference***
MTL-CEBPA normalises serum albumin

**Pre treatment**  
Week 8

**Post treatment**  
Week 10

### Albumin (g/dL)

**Pre treatment**
- Normal control
- NOV340 +siFLUC
- MTL-CEBPA

**Post treatment**
- Normal control
- NOV340 +siFLUC
- MTL-CEBPA

+CCl4 dosing
MTL-CEBPA normalises prothrombin time

Pre treatment
Week 8

Post treatment
Week 10

Prothrombin Time (s)

Normal control
NOV340 +siFLUC
MTL-CEBPA

Normal control
NOV340 +siFLUC
MTL-CEBPA

+CCI4 dosing

+CCI4 dosing

***

MTL-CEBPA normalises prothrombin time.
MTL-CEBPA attenuates hyper-ammonaemia

**Pre treatment**
Week 8

**Post treatment**
Week 10

Ammonia (µmol/L)

<table>
<thead>
<tr>
<th>Normal control</th>
<th>NOV340 +siFLUC</th>
<th>MTL-CEBPA</th>
</tr>
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<td>+CCI4 dosing</td>
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**Ammonia (µmol/L)**

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MTL-CEBPA normalises serum AST

<table>
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<tr>
<th>Group</th>
<th>Week 8 (Pre treatment)</th>
<th>Week 10 (Post treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>NOV340 + siFLUC</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>MTL-CEBPA</td>
<td>600</td>
<td>200</td>
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</table>

+CCI4 dosing

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<td>MTL-CEBPA</td>
<td>600</td>
<td>200</td>
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+CCI4 dosing
MTL-CEBPA normalises serum ALT

**Pre treatment**
Week 8

**Post treatment**
Week 10

- Normal control
- NOV340 + siFLUC
- MTL-CEBPA

+ CCl4 dosing

**ALT (U/L)**

Normal control NOV340 MTL-CEBPA

100 200 300 400 500

Normal control NOV340 MTL-CEBPA

100 200 300 400 500

***
MTL-CEBPA normalises serum ALP

Pre treatment
Week 8

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALP (U/L)</th>
</tr>
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<tbody>
<tr>
<td>Normal control</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>NOV340 + siFLUC</td>
<td>200 ± 10</td>
</tr>
<tr>
<td>MTL-CEBPA</td>
<td>300 ± 15</td>
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</table>

Post treatment
Week 10

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALP (U/L)</th>
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<tbody>
<tr>
<td>Normal control</td>
<td>50 ± 2</td>
</tr>
<tr>
<td>NOV340 + siFLUC</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>MTL-CEBPA</td>
<td>200 ± 10</td>
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+CCI4 dosing
MTL-CEBPA normalises body weight

Pre treatment
Week 8

Post treatment
Week 10

MTL-CEBPA normalises body weight
Clinical track record of MTL-CEBPA liposomal formulation SMARTICLES

**MRX34**
- Well tolerated at 110 mg/m² in ongoing Phase I study in liver cancer and hematological malignancies
- 9 mg/kg NOAEL identified in NHP toxicology study
- Tumour regression in mouse model of HCC at 0.3 mg/kg

**PNT2258**
- Anti-tumour activity at 120 mg/m² in Phase II in non-Hodgkin’s Lymphoma
- Well tolerated in Phase I up to 150 mg/m²
**OUTReACH**
Phase 1 in HCC with impaired liver function

<table>
<thead>
<tr>
<th>First patient in</th>
<th>Q1 2016</th>
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<td><strong>Design</strong></td>
<td>Open label, First in Human dose escalation in cohorts of 3 patients</td>
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<tr>
<td><strong>Indications</strong></td>
<td>Advanced tumour diseases with low serum albumin levels, characterised by primary or secondary liver tumours</td>
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| **Objectives**   | Primary: To determine the safety of administering MTL-CEBPA to patients with liver tumours and low serum albumin  
                   Secondary: To determine the RP2D; characterise the PK of MTL-CEBPA; characterise the PD of MTL-CEBPA; to increase serum albumin and/or decrease serum bilirubin |
| **Administration**| 60min I.V. infusion  
                   QWx3 + 1 week rest (4 week cycle) |
| **UK centres**   | King's College London  
                   Imperial College London  
                   UCL  
                   Newcastle University  
                   University of Liverpool  
                   University of Cambridge |
• MiNA Therapeutics developing short activating RNA compounds to selectively up-regulate gene expression

• MTL-CEBPA candidate targets CEBPA gene promoter for increased C/EBP-α expression

• MTL-CEBPA reverses CCl4 induced liver failure in vivo

• OUTREACH Phase I study initiating in Q1 2016